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FORMATIVE EFFECT OF HIGH AND LOW TEMPERATURES UPON GROWTH OF BARLEY: A CHEMICAL CORRELATION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 259

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(WITH EIGHTEEN FIGURES)

Introduction

Cereals are commonly considered cool temperature crops. Cool seasons are known to favor cereal production, warm seasons to hinder cereal production. Physiologists have correlated these observations with the general effects of temperature upon the growth and maturation of the crop, but have given little attention to possible effects of the initial germination temperature upon the subsequent course of development of the plant. The investigation here reported is a study of the effects of high and low temperatures and concomitant variations in the supply of nitrogen, phosphorus, and potassium respectively upon the course of development of the barley plant. A chemical correlation has been established between temperature and nutrition effects.

Literature

ADERHOLD (1), working with young kohlrabi plants, noted that exposures of the young plants to temperatures of $-2°$ C. to $-8°$ C. for 8–10 hours tended to cause the plant to shoot into flowering instead of forming the desired "ball."

GUTZEIT (5) repeated ADERHOLD'S work and found by a rather extensive set of experiments that exposures to temperatures below zero had no effect on stem or shoot production in kohlrabi, beets, or various other plants. He did find, however, that a period at +4° C. during germination and early growth caused about 30 per cent of certain beets to produce shoots very early the first year. Some of the shoots produced only very short stems, and the plants were otherwise normal, while other shoots grew continuously and early produced flowers and seeds. Beets of exactly the same kind when kept at +22° C. during germination and early growth showed no shoot production the first year. Only such beets as were predisposed to early shoot production could be thus forced by low temperatures, so hereditary characters as well as temperature enter in as determining factors. GUTZEIT suggests that this temperature response explains why early seeding of beets causes much premature shoot production, whereas late seeding gives little or none. On the basis of other experiments conducted by himself, as well as data from the literature, GUTZEIT concludes that low temperatures during germination and early growth favor stem formation, while high temperatures at this time inhibit stem formation.

APPEL and GASSNER (2) noted in the experimental fields of summer cereals at the Agricultural Experiment Station at Dahlem, Germany, a peculiar sickness, the plants becoming light green, and the older leaves turning yellow. Since neither animal nor plant pests seemed to be attacking the cereals, an explanation for their condition was sought in unfavorable soil and weather relations. Greenhouse experiments conducted by APPEL and GASSNER led them to attribute the peculiar conditions of these summer cereals to a too high germination temperature.

They grew barley in pots in the greenhouse, keeping one lot at 20–25° C. and the other lot at 5–7° C. When the plants at the higher temperature had reached a height of 15 cm., those in the cool house had just come up. Both sets were then transferred to the open and kept under like conditions. After three weeks the barley plants from the warm house began to show signs of injury, the older leaves yellowing at their tips, and only the youngest leaves remaining green. The barley plants from the cool house

soon outstripped the high temperature plants, finally reaching twice the size. Figures of APPEL and GASSNER'S plants show that there was an excessive leaf production and little stem production at the higher temperature. These investigations suggested that the light color of the leaves was due to nitrogen hunger, but they were unable to get any beneficial results from nitrogen fertilization. The addition of iron salts also had no favorable effect.

GASSNER (3) has made extensive observations and experimental studies upon the growth and development of cereals in subtropical climates, the experiments being carried out in the phytopathological experimental fields of the University of Montevideo, Uruguay. In considering the choice of varieties of summer cereals suitable for cultivation in Uruguay, he emphasizes the importance of temperature in the early stages of development, and suggests that decreased yields are often due to the lack of the necessary cold requirements (Kälteansprüche) in the early stages of growth. GASSNER quotes HELLRIEGEL (6) on the temperature relations of small 4-rowed barley. HELLRIEGEL maintained that in the first half of the vegetative period of the barley, the period of leaf and culm formation, an average daily temperature of about 15° C. is the best, whereas in the second half of the vegetative period, the period of head development and grain formation, a temperature of 17–18° C. is the most favorable. HELLRIEGEL therefore insists upon two different temperature optima in development of barley, the line of demarcation between the two optima being placed at the time of shooting.

GASSNER summarizes his views as follows (translated from the original article):

> We can therefore say that for winter cereals, as well as for summer cereals, the yield of a given variety of a cereal in a given climate is among other things dependent upon the influence of the climatic factors in the first stage of development in such a way that varieties of high "cold requirements" in their youth require a colder climate than varieties with lower "cold requirements," and that incomplete fulfillment of these requirements causes bad development and depression of the yield.

GASSNER states that the death and yellowing of the leaves of young plants previously described by APPEL and GASSNER rarely

occurs in Uruguay. He notes, however, that the culm habit in Uruguayan oats and rye germinated at high temperatures is distinctly recumbent, whereas it is upright from the beginning in the case of plants grown from seeds germinated at low temperatures. The low temperature plants begin the formation of the culm (shooting) much earlier than do the high temperature plants. A typical experiment with oats is outlined as follows:

Date of seeding	Temperature during germination	Date of transfer into field	Beginning shooting
January 18	January 18–23, 6–9°; January 23–25, 25°	January 25	March 15
January 18	January 18–20, 25°; January 20–25, 6–9°	January 23	No shoot formation on April 25, shooting not expected before October

In another series it was found that even 24 hours of exposure to a germination temperature of 25° led to the same abnormal course of development as indicated in the second series here quoted.

GASSNER and GRIMME (4) have made one attempt to correlate the effects of germination temperatures and the resistance of cereals to frost injury. They analyzed the first leaves of winter and spring rye germinated at 5–6° C. and at 28°. They found that the seedlings germinated at the lower temperature had a higher sugar content than seedlings germinated at the high temperature; moreover, seedlings of a hardy winter rye had a higher sugar content than those of a spring rye grown under the same conditions. Their results with rye are shown in table I.

HUTCHESON and QUANTZ (7) conducted experiments on the effect of greenhouse temperatures on the growth of the small grains: wheat, oats, barley, and rye. All four crops were grown under four temperature conditions, namely, 14.4° C., 16.6° C., 18.3° C., and 23.9° C. The higher temperature range had a distinctly detrimental effect upon the growth of the barley and a less harmful effect upon the growth of wheat and rye, while oats had a normal course of development at all the temperatures used, although the oat culms were weaker at the higher temperatures. The high

temperature barley plants showed an excessive development of tillers and no indication of ever heading. Inspection of the figures shows that the leaves of the high temperature plants were abnormally long, and especially so in the case of the barley. The general growth characters obtained by HUTCHESON and QUANTZ were obtained in the present investigation in the case of high temperature, high nitrogen series (fig. 13). These authors grew the grain

TABLE I

SUGAR CONTENT OF FIRST LEAVES OF RYE* (PERCENTAGE OF DRY WEIGHT)

SERIES NO.	TOTAL SUGAR	GERMINATION TEMPERATURE 5–6° C.		GERMINATION TEMPERATURE 28° C.		
		Reducing sugar	Non-reducing sugar	Total sugar	Reducing sugar	Non-reducing sugar
	Petkuser winter rye					
I	42.19	34.93	7.26	40.92	32.56	8.36
II	43.14	35.86	7.28	39.79	31.14	8.65
III	41.92	34.84	7.08	39.13	31.08	8.05
IV	42.31	35.85	6.46	40.73	33.94	6.79
V	40.97	32.31	8.66	39.52	34.11	5.41
	Petkuser spring rye					
I	36.58	29.41	7.17	31.57	27.13	4.44
II	37.08	30.57	6.51	33.26	26.58	4.68
III	35.39	30.41	4.98	32.59	26.81	5.78
IV	37.65	31.02	6.63	34.56	30.38	4.18
V	35.85	30.21	5.64	32.94	28.16	4.78

* Similar results were obtained with barley.

in 4-inch clay pots, two plants to the pot. No mention is made concerning the substrate used in their experiments.

This investigation of the influence of high and low temperatures upon the growth of barley was planned to ascertain in particular the influence of variations in the supply of nutrient salts with concomitant variations in the temperature. The nutrients varied were nitrogen, potassium, and phosphorus. Chemical analyses were made in order to relate certain observed differences in growth to possible differences in the chemical composition.

Method

Culture solutions

The method of sand culture was used throughout these experiments, the sand used being a highly pure Ottawa silica sand obtained from Ottawa, Illinois. Two gallon glazed stone jars were used as the culture vessels, each jar receiving 11.4 kilos of sand. The water content of each jar was maintained at approximately 13 per cent of the dry weight of the sand by means of frequent weighing. Tottingham's culture solution was used in diluted form. This solution has the following composition:

Solution A: $\begin{cases} KNO_3 - 0.0034\ M\ (0.3437\ \text{gm. per liter}) \\ KH_2PO_4 - 0.0108\ M\ (1.4692\ \text{gm. per liter}) \\ MgSO_4 - 0.0081\ M\ (0.9750\ \text{gm. per liter}) \end{cases}$

Solution B: $Ca(NO_3)_2 - 0.0101\ M$ (1.6573 gm. per liter)

Enough of these salts to make 100 liters of culture solution were dissolved and made up to 2 liters, the $Ca(NO_3)_2$ being made up in a separate 2-liter portion in order to prevent precipitation of insoluble calcium salts in the highly concentrated solution. The mixture of these two solutions was designated solution A B, and 7.5 cc. of each of these solutions were added to 1500 cc. of distilled water for the initial dose of nutrient solution. This quantity of nutrient solution was applied to the jars designated in the outline of the scheme of the experiment at the time of planting (March 1). In addition, 0.01 gm. of $FeCl_3$ was added to each culture one week after sowing. On April 4 each A B culture received a second dose of 7.5 cc. of this normal nutrient solution. All cultures receiving only A B solutions will be referred to hereafter as "normal."

Solutions lacking in P, N, and K were also made. The amount of salts indicated in the respective tables were dissolved and made up to 2000 cc. with distilled water; 75 cc. of these solutions made up to 1500 cc. were used as initial doses. Similarly, solutions were made up in which the P, N, and K were supplied in one-fourth the concentration of that found in solution A B.

Solution C
(lacking in phosphorus)

3.437 gm. KNO_3
8.8561 gm. KCl
23.855 gm. $Ca(NO_3)_2$, $4H_2O$
19.789 gm. $MgSO_4$, $7H_2O$

Solution D
(phosphorus in one-fourth concentration)

3.437 gm. KNO_3
3.873 gm. KH_2PO_4
6.642 gm. KCl
23.855 gm. $Ca(NO_3)_2$, $4H_2O$
19.789 gm. $MgSO_4$, $7H_2O$

Solution E
(lacking nitrogen)

2.723 gm. KCl
14.6923 gm. KH_2PO_4
12.353 gm. $CaCl_2$, $2H_2O$
19.789 gm. $MgSO_4$, $7H_2O$

Solution F
(nitrogen in one-fourth concentration)

0.8592 gm. KNO_3
2.0422 gm. KCl
14.6923 gm. KH_2PO_4
19.7890 gm. $MgSO_4$, $7H_2O$
4.1432 gm. $Ca(NO_3)_2$, $4H_2O$
9.264 gm. $CaCl_2$, $2H_2O$

Solution G
(lacking potassium)

2.8894 gm. $NaNO_3$
17.0692 gm. Na_2HPO_4, $12H_2O$
23.855 gm. $Ca(NO_3)_2$, $4H_2O$
19.789 gm. $MgSO_4$, $7H_2O$

Solution H
(potassium in one-fourth concentration)

0.8592 gm. KNO_3
2.1671 gm. $NaNO_3$
3.873 gm. KH_2PO_4
12.8019 gm. Na_2HPO_4, $12H_2O$
19.789 gm. $MgSO_4$, $7H_2O$
23.8558 gm. $Ca(NO_3)_2$, $4H_2O$

To certain of the A B cultures extra doses of N, K, and P, alone, and in all possible combinations, were added in the amounts and at the times indicated in the schematic outline. These extra doses were supplied in the form of solutions of $NaNO_3$, KCl, and Na_2HPO_4, $12H_2O$ respectively. All cultures were run in triplicate. Certain of the replicates in each set of triplicates received a modified supplementary treatment, as indicated in table II, the letters N, K, and P indicating $NaNO_3$, KCl, and Na_2HPO_4, $12H_2O$ respectively.

Oderbrucker barley (Wisconsin No. 5) was seeded March 1. About 30 seeds were sown per culture, the cultures being thinned to 25 plants per culture. This heavy seeding was purposely chosen in order to prevent tillering, so that the course of development of a plant with a single culm could be followed.

TEMPERATURE AND HUMIDITY CONTROL.—The temperature of the greenhouses was controlled by means of automatic thermoregulators. The lower temperature selected was 15° C., the higher temperature 20° C. The degree of control obtained is shown in

TABLE II

OUTLINE OF GREENHOUSE EXPERIMENTS

Jar no.*	General treatment	Supplementary treatment
1, 2, 3 64, 65, 66	Distilled water only	....
4, 5 68, 69	Solution C	....
6 67	Solution C	2 gm. P added April 27
7, 8, 9... 70, 71, 72	Solution D	1 gm. N added April 26
10, 11, 12 73, 74, 75	Solution A B	1 gm. N added April 26
13, 14, 15 76, 77, 78	Solution A B+1 gm. P	Second dose of 1 gm. of P added March 30
16, 17 80, 81	Solution E	....
18 71	Solution E	4 gm. N added April 27
19, 21 82, 84	Solution F	....
20 83	Solution F	4 gm. N added April 27
22, 23 85, 86	Solution A B+1 gm. N	Second dose of 1 gm. of N on March 20; third dose of 2 gm. on April 26; fourth dose of 2 gm. on April 29
24 87	Solution A B+1 gm. N	Second dose of 1 gm. of N on March 30
25, 26, 27 88, 89, 90	Solution A B	1 gm. N added April 26
28, 30 91, 92	Solution G	1 gm. N added April 27
29 93	Solution G	1 gm. N added April 27; 2 gm. K added April 27
31, 32, 33 94, 95, 96	Solution H	1 gm. N added April 27
34, 35, 36 97, 98, 99	Solution A B	1 gm. N added April 27
37, 38, 39 100, 101, 102	Solution A B+1 gm. KCl	Second dose of 1 gm. K March 30; 1 gm. N April 26
40, 42 103, 104	Solution A B+1 gm. N, 1 gm. P	Second dose of 1 gm. N and 1 gm. P March 30; third dose of 2 gm. of each April 26; 2 gm. N only April 29
41 105	Solution A B+1 gm. N, 1 gm. P	Second dose of 1 gm. N and 1 gm. P March 30; 1 gm. N and 2 gm. P April 26
43, 45 106, 107	Solution A B+1 gm. N	Second dose of 1 gm. N March 30; third dose of 1 gm. N April 26
44 108	Solution A B+1 gm. N	Second dose of 1 gm. N April 26
46, 47, 48 109, 110, 111	Solution A B+1 gm. P, 1 gm. K	Second dose of 1 gm. of P and 1 gm. K March 30; 1 gm. N April 26
49, 50	Solution A B+1 gm. N	Second dose of 1 gm. N and 1 gm. K March 30; third dose of 2 gm. of each April 26; 2 gm. more of N April 29

TABLE II—*Continued*

Jar no.	General treatment	Supplementary treatment
51, 114	Solution A B+1 gm. N, 1 gm. K	Second dose of 1 gm. N and 1 gm. K March 30; 1 gm. N and 2 gm. K April 26
52, 53, 54, 115, 116, 117	Solution A B+1 gm. P	Second dose of 1 gm. P March 30; 1 gm. N April 26
55, 56, 119, 120	Solution A B+1 gm. N, 1 gm. K, 1 gm. P	Second dose of 1 gm. each of N, K, and P March 30; of 2 gm. each April 26; 2 gm. N April 29
57, 118	Solution A B+1 gm. N, 1 gm. K, 1 gm. P	Second dose of 1 gm. each of N, K, and P March 30; 1 gm. N and 2 gm. each of K and P April 26
58, 59, 122, 123	Solution A B+1 gm. N, 1 gm. P	Second dose of 1 gm. each of N and P March 30; 2 gm. more of each April 26; 2 gm. of N April 29
60, 121	Solution A B+1 gm. N, 1 gm. P	Second dose of 1 gm. each of N and P March 30; 1 gm. N and 2 gm. P April 26
61, 62, 124, 125	Solution A B+2 gm. P, 1 gm. N	Second dose of 2 gm. P and 1 gm. N March 30; 4 gm. P and 2 gm. N April 26; 2 gm. N April 29
63, 126	Solution A B+2 gm. P, 1 gm. N	Second dose of 2 gm. P and 1 gm. N March 30; 4 gm. P and 1 gm. N April 26

* Jars nos. 1–63 inclusive kept in warm greenhouse; jars nos. 64–126 inclusive kept in cool greenhouse

the thermograph records obtained in the two houses (figs. 1, 2). It will be noted that there was a fairly satisfactory degree of control up to about the middle of April, at which time (April 19) the samples for chemical analyses were taken. The principal fluctuations came at about noon; a considerable temperature difference always existed.

The degree of humidity was not under a complete control as desired, the evaporation rate averaging somewhat higher in the warm house. It is possible that some of the differences noted in chemical composition are due to the higher evaporating power of the air in the warm house. This higher evaporation rate was, of course, a function of the higher temperature.

Observations on growth of barley cultures

During the first two weeks of growth the plants in the warm house, which were several inches high before the plants in the cool house had come up, maintained a more rapid growth rate. The first leaves of all of the plants in the warm house, except those receiving little or no nitrogen, tended to lop over. The low

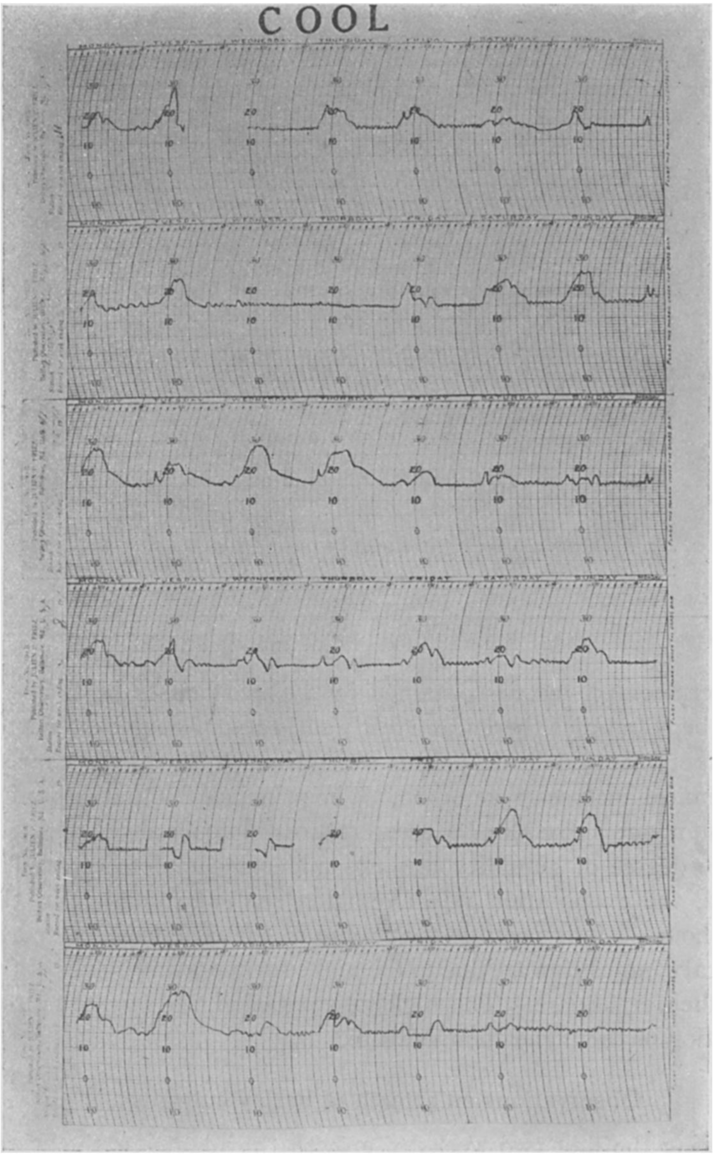

FIG. 1.—Thermograph records showing air temperature in cool house from planting to time of sampling for chemical analysis

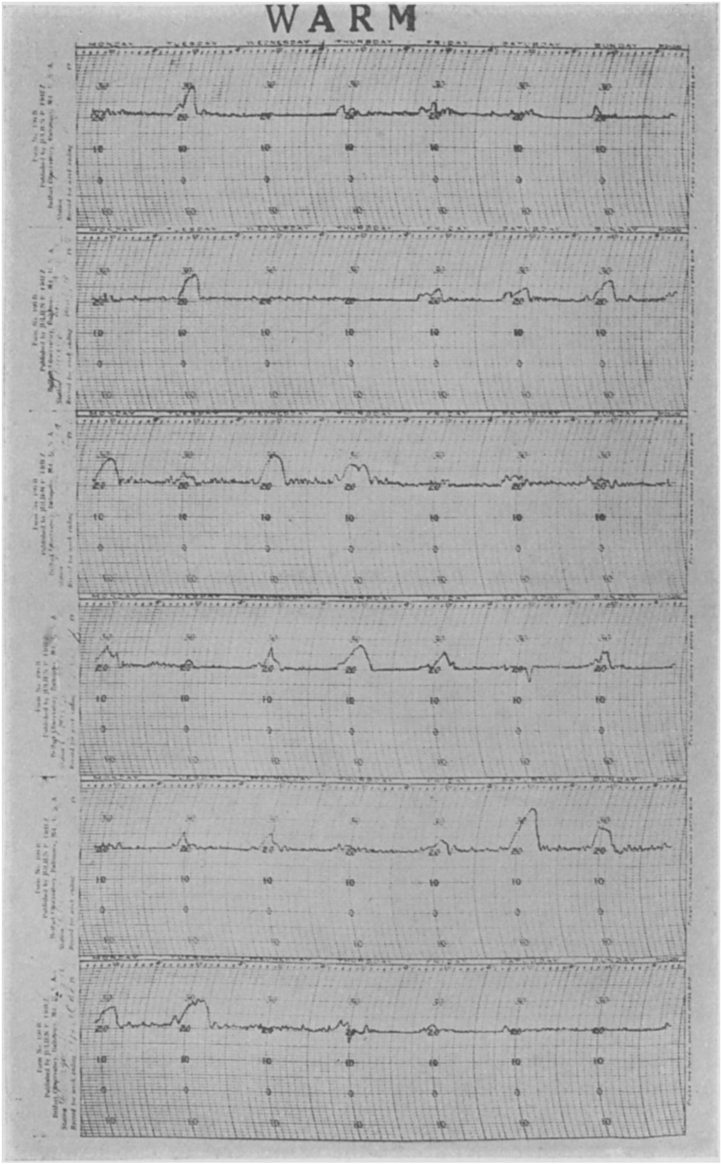

FIG. 2.—Thermograph records showing air temperature in warm house from planting to time of sampling for chemical analysis

nitrogen leaves were in every case stiff and upright. By March 16 the "no phosphorus" series began to show the effect of the deficiency. The "no potassium" series in the warm house showed the

TABLE III

PROPORTION OF LEAVES (BLADE AND SHEATH) AND STEMS IN 100 PARTS OF TOTAL PLANTS, BASED ON GREEN WEIGHT

Culture no. and treatment	Leaves (blades and sheaths) Percentage	Stems Percentage
44. High N warm	92.95	7.05
24. High N warm	88.23	11.73
40. High P and N warm	96.87	3.23
108. High N cool	69.20	30.80
87. High N cool	65.68	34.32
104. High P and N cool	71.37	28.63

greatest lopping over on March 16. About April 1 the plants in the cool house began to outstrip the plants in the warm house in their growth rate, and in particular in their tendency to maintain an upright growth habit. The total amount of tissue formed at

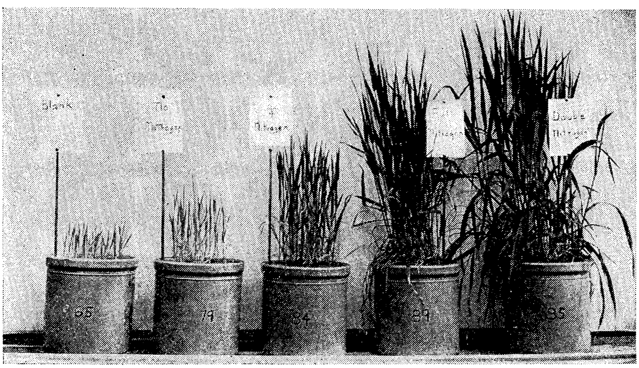

FIG. 3.—Nitrogen series, cool house: note vigorous upright condition of no. 85 as compared with sprawling condition of no. 23 (fig. 4).

the higher temperature was about the same, but it was differently distributed, as will be apparent from the data given in table III.

By April 19 all plants in the cool house had outstripped those in the warm house. The most striking difference between the two

houses was the sprawling condition of the high nitrogen cultures in the warm house, in contrast with their upright condition in the cool house. Figs. 3–15, taken April 24, show the condition of the barley on that date.

Fig. 4.—Nitrogen series, warm house

Fig. 5.—Phosphorus series, cool house: N and K treatment of nos. 68, 71, 75, and 78 "normal" (same as no. 89 in fig. 3).

Figs. 16–18, taken May 16, show the failure of the high nitrogen-high temperature plants to mature normally. Such shooting as was obtained at the higher temperature was due, in the opinion of the writer, to inability to control the moisture supply, because of very great fluctuation in the temperature as the spring season advanced. The writer believes that had it been possible to control absolutely

temperature and moisture supply, the high nitrogen-high temperature series could have been maintained in practically continuous vegetation without any tendency to reproduce. The reason for this belief was the failure of this series to produce any stem (culm) until the water supply fell below the normal previously maintained.

Chemical examination of tissues

In order to ascertain, if possible, the character of the internal processes that determine this very striking formative effect of the

FIG. 6.—Phosphorus series, warm house: N and K treatment of nos. 6, 8, 12, and 15 "normal" (same as no. 25 in fig. 4).

higher temperature in the presence of high nitrogen supply, tissue analyses were made on the leaves (blades plus sheaths) from 100 gm. of total plants from cultures nos. 44, 24, 108, 87, and 104. The plants were selected so that the sample equaled 100 gm. Table III shows the very low percentage of stem material at the higher temperature. Since both leaf-blade and leaf-sheath are active organs in cereals, both were included. The second column in the table shows the green weight in grams of this leaf tissue. The first column in table VI shows the date and hour of taking these samples.

Methods of tissue analysis

The green samples were weighed and immediately preserved by adding enough ethyl alcohol to make a 75 per cent alcoholic solution, and then boiled to arrest enzymic activity. The preserved

Fig. 7.—Potassium series, cool house: N and P treatment of nos. 92, 94, and 100 same as no. 89 in fig. 3; note sprawling condition of "no potash" culture.

Fig. 8.—Potassium series, warm house: N and P treatment of nos. 29, 32, and 38 same as no. 25 in fig. 4.

material was then subjected to the method of tissue analysis devised by Waldemar Koch, and modified by F. C. Koch (**8**). The method used consisted essentially of 4 hours' extraction with hot ethyl alcohol in a continuous extractor, followed by 1 hour's

extraction with ether, then treatment of the finely ground material with hot water several times, after which the aqueous mixture was made up to a concentration of 75 per cent alcohol and filtered. The insoluble material was then subjected to further extraction with hot alcohol for 24 hours.

The combined extractions were evaporated to dryness on a steam bath, then repeatedly evaporated with absolute ethyl alcohol in order to remove water. The dry hard residue was then

FIG. 9.—Effect of heavy N fertilization: no. 12, normal N (warm house); no. 85, heavy N (cool house); no. 22, heavy N (warm house); no. 75, normal N (cool house).

extracted with anhydrous ether by grinding with a pestle with successive portions of fresh ether. The ethereal extracts were made up to 250 cc., and then divided into suitable aliquots for chemical and dry weight determinations (50 cc. portions). This extraction was designated as fraction 1 (F_1). The ether-insoluble residue was taken up in about 65 per cent alcohol and made up to a volume of 500 cc., 50 cc. portions being taken as aliquots for analysis and dry weight determinations. This was designated as fraction 2 (F_2). Moisture determinations were made on duplicate F_1 and F_2 aliquots

by evaporating almost to dryness on the steam bath and then taking down to constant weight in a vacuum desiccator.

TABLE IV

EFFECT OF TEMPERATURE UPON AMOUNT AND PERCENTAGE OF DRY MATTER AND WATER IN BARLEY LEAVES

Culture no. and treatment	Green weight (gm.)	Dry weight (gm.)	Weight of water (gm.)	Percentage of water	Percentage of dry matter
44. High N warm........	92.95	12.67	80.28	86.36	13.64
24. High N warm........	88.23	12.79	75.44	85.50	14.50
41. High P and N warm...	96.87	11.10	85.77	89.47	10.53
108. High N cool.........	69.20	10.92	58.28	84.21	15.79
87. High N cool.........	65.68	10.23	55.45	84.42	15.58
104. High P and N cool....	71.37	11.04	60.23	84.39	15.61

Material insoluble in ether, alcohol, and water was designated as fraction 3 (F_3). This entire fraction was dried to constant weight

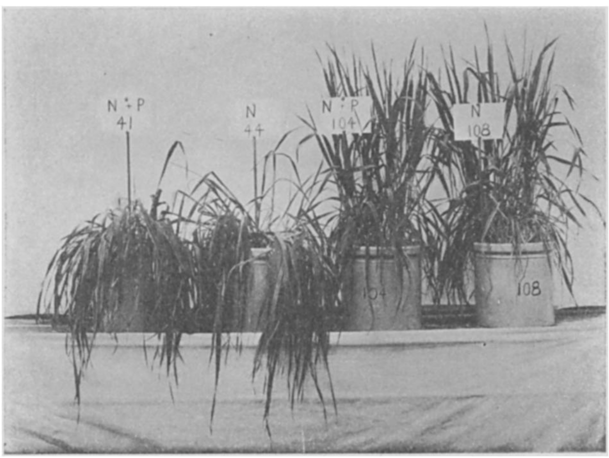

FIG. 10.—Influence of supplementary P fertilization on heavy N fertilization: all cultures received equal heavy doses of N in form of $NaNO_3$; cultures nos. 41 and 104 received equal dosage of extra P; nos. 41 and 44 grown in warm house; nos. 104 and 108 in cool house; P failed to counteract effects of N at higher temperature; chemical analyses made of leaves from this set of cultures.

at 100° C. in an electrically heated oven. Table IV gives the relative proportions of moisture and dry matter in the several samples analyzed.

Table V gives the distribution of the several fractions in the samples analyzed. Particular attention is directed to the fact that the temperature does not seem to have any important effect upon the proportion of lipins (F_1), except where extra phosphorus is present, in which case a high temperature led to an increase in the lipin material. The author regrets not being able to confirm this

FIG. 11.—Influence of supplementary K fertilization on heavy N fertilization: nos. 50 and 113 received equal heavy doses of N in form of $NaNO_3$; nos. 100 and 38 received only "normal" N; all 4 cultures received equal heavy doses of K in form of KCl; nos. 50 and 38 warm house; nos. 100 and 113 cool house; K failed to counteract effects of N at higher temperature.

interesting observation by means of further analyses. The proportion of fraction 2, which might quite properly be designated the metabolic fraction, averages about 10 per cent higher at the higher temperature. The proportion of fraction 3, or storage and skeleton fraction, averages nearly 8 per cent higher at the lower temperature.

F_1 was analyzed for total N and total P. F_2 was analyzed for total N (organic and ammoniacal only), total P, direct reducing

sugars, and for total sugars after mild hydrolysis. Samples 24 and 87 were also analyzed for inorganic phosphorus, using the POWICK-CHAPIN (10) method. F_3 was analyzed for total N, total P, N and P soluble and insoluble in 1 per cent NaOH, phosphoprotein phosphorus, polysaccharides, and cellulose, etc., by

TABLE V

EFFECT OF TEMPERATURE ON DISTRIBUTION OF EXTRACTIVES AND INSOLUBLE MATTER IN BARLEY LEAVES

Culture no. and treatment	Soluble in anhydrous ether (F_1) Percentage	Soluble in hot alcohol and water (F_2) Percentage	Insoluble in ether, alcohol, and water (F_3) Percentage
44. High N warm	8.699	33.017	58.284
24. High N warm	7.885	33.743	58.372
41. High P and N warm	10.433	32.613	56.954
108. High N cool	8.251	30.321	61.428
87. High N cool	8.663	27.823	63.514
104. High P and N cool	7.681	30.279	62.040

difference. The following list gives the methods employed. The details of the several methods are those recommended by KOCH (8) and MATHEWS (9).

Total nitrogen..........Arnold-Gunning method.
Total phosphorus.......Neuman-Pemberton method.
Direct reducing sugars...Bertrand volumetric method (glucose calculated from Munson-Walker tables in MATHEW'S *Physiological Chemistry*).
Total sugars............Bertrand volumetric method applied to the products of mild hydrolysis with HCl.
Polysaccharides.........Bertrand volumetric method applied to the products of strong hydrolysis with HCl.
Phosphoprotein phosphorus..........Determination of the P precipitable by Mg mixture in an extract made by 48 hours' digestion with 1 per cent NaOH at 37–40° C.

The method for phosphoprotein phosphorus is based upon the discovery by PLIMMER and SCOTT that phosphoproteins can be separated from nucleoproteins through hydrolyzing the former with 1 per cent NaOH, the latter being unattacked by the dilute

alkali. The exact details of the method used on this material are as follows. Weighed samples of F_3 were placed in 300 cc. Erlenmeyer flasks, usually about 0.5 gm., and 1 per cent NaOH, free from phosphorus, was then added at the rate of 100 cc. of NaOH for each 1.0 gm. of substance. The flasks were stoppered and placed in an electric incubator at 37–40° C., where they were allowed

FIG. 12.—Influence of supplementary fertilization with both K and P on heavy N fertilization: no. 120, heavy N+extra K and P, cool house; no. 47, "normal" N+extra K and P, warm house; no. 55, heavy N+extra K and P, warm house; no. 110, "normal" N+extra K and P, cool house; note that "complete fertilizer" failed to counteract effects of heavy N at higher temperature; are not growth effects noted in no. 55 referable to stimulus received at time of germination?

to remain 48 hours. The flasks were shaken about 4 times each day. At the end of the digestion period the insoluble material was filtered off on ashless filter papers and carefully washed with lukewarm water. The combined filtrate and washings were then neutralized to litmus with acetic acid and the PO_4 ions precipitated with magnesia mixture in the presence of an excess of NH_4OH. This precipitation was conducted at a low temperature, the solutions

being allowed to stand in the ice box for 24 hours. At the end of the 24 hour period the magnesium ammonium phosphate was filtered off, washed with 2.5 per cent cold ammonia water, dissolved

FIG. 13.—Influence of variation in fertilization in warm house: N, K, and P indicate that fertilizer dosage is in excess of "normal" A B solution; contrast with results shown in fig. 14, where fertilizer treatment is identical but temperature lower.

FIG. 14.—Influence of variation of fertilization in cool house: contrast with fig. 13

in dilute nitric acid, and the phosphorus then precipitated by means of the molybdate solution. Final determination of the phosphorus was made by means of the Pemberton alkalimetric method.

In order to determine whether or not the material thus extracted by 1 per cent NaOH contained any forms of P not precipitated by magnesia mixture, the phosphorus was determined in the insoluble residue. Similarly total N determinations were made in the insoluble residue from another set of determinations. The difference

FIG. 15.—Influence of extra heavy supplementary P fertilization on heavy N fertilization: no. 41, heavy N and heavy P (warm house); no. 125, heavy N and extra heavy P (cool house); no. 104, heavy N and heavy P (cool house); no. 63, heavy N and extra heavy P (warm house).

between total P (or N) soluble in 1 per cent NaOH gave the P (or N) present in the NaOH extract.

TABLE VI

EFFECT OF TEMPERATURE ON ACCUMULATION OF SOLUBLE CARBOHYDRATES IN BARLEY LEAVES (RESULTS OF ANALYSIS OF F_2)

Culture no., treatment, and time of sampling	Direct reducing sugars (as glucose)		Total sugars after mild hydrolysis (as glucose)		Percentage concentration of total sugars (as glucose) in total water in tissue
	Percentage F_2	Percentage total leaf	Percentage F_2	Percentage total leaf	
44. High N 4 P.M., April 24, 1918 warm.......	15.99	5.27	29.41	9.71	0.15252
24. High N 9 A.M., April 25, 1918 warm.......	19.83	6.69	26.84	9.05	0.15342
41. High P and N 3 P.M., April 25, 1918 warm..	10.62	3.46	18.55	5.05	0.06535
108. High N 6 P.M., April 24, 1918 cool.........	20.20	6.11	45.79	13.88	0.26007
87. High N 10 A.M., April 24, 1918 cool........	23.17	6.44	36.99	10.29	0.18985
104. High P and N 5 P.M., April 24, 1918 cool...	25.08	7.59	39.90	12.08	0.22142

Tables VI–XII contain the results of the different determinations. Table XIII gives the proportion of skeletal material in

F_3, calculated as the difference between the total amount of the fraction, and the sum of the protein and starch in the fraction.

TABLE VII

EFFECT OF TEMPERATURE ON ACCUMULATION OF POLYSACCHARIDES IN BARLEY LEAVES

Culture no. and treatment	Percentage of F_3	Percentage of total leaf
44. High N warm	21.16	12.33
24. High N warm	21.64	12.63
41. High P and N warm	20.43	11.63
108. High N cool	25.51	15.67
87. High N cool	23.60	14.99
104. High P and N cool	24.14	14.97

TABLE VIII

EFFECT OF TEMPERATURE ON AMOUNT OF NITROGEN, PHOSPHORUS, PROTEIN, AND PHOSPHOPROTEIN PHOSPHORUS IN BARLEY LEAVES (PERCENTAGE OF TOTAL DRY WEIGHT)

Culture no. and treatment	Total N	Total P	Total protein (Percentage N in $F_3 \times 6.25$)	Phosphoprotein phosphorus
44. High N warm	2.9779	0.6944	12.965	0.1167
24. High N warm	2.7007	0.6789	11.277	0.1411
41. High P and N warm	3.4661	0.7988	14.425	0.0795
108. High N cool	2.5610	0.5479	12.865	0.0543
87. High N cool	2.6065	0.5711	13.159	0.0832
104. High P and N cool	2.5945	0.6532	12.008	0.0996

TABLE IX

EFFECT OF TEMPERATURE ON AMOUNT AND DISTRIBUTION OF NITROGEN IN BARLEY LEAVES

CULTURE NO. AND TREATMENT	F_1 SOLUBLE IN ANHYDROUS ETHER			F_2 SOLUBLE IN HOT ALCOHOL AND WATER			F_3 INSOLUBLE IN ALCOHOL, ETHER, OR WATER		
	Percentage fraction	Percentage total leaf	Percentage total N in leaf	Percentage fraction	Percentage total leaf	Percentage total N in leaf	Percentage fraction	Percentage total leaf	Percentage total N in leaf
44. High N warm	1.4993	0.1304	4.38	2.3420	0.7732	25.96	3.559	2.0743	69.66
24. High N warm	1.3081	0.1021	3.78	2.1312	0.7191	26.62	3.237	1.8795	69.60
41. High P and N warm	2.6700	0.2785	8.03	2.6970	0.8795	25.377	4.053	2.3081	66.60
108. High N cool	1.4142	0.1167	4.55	1.2730	0.3859	15.07	3.351	2.0584	80.38
87. High N cool	1.2520	0.1084	4.16	1.4110	0.3925	15.06	3.315	2.1054	80.78
104. High P and N cool	1.3205	0.1014	3.91	1.4130	0.4278	16.49	3.329	2.0653	79.60

TABLE X

EFFECT OF TEMPERATURE ON AMOUNT AND DISTRIBUTION OF PHOSPHORUS IN BARLEY LEAVES

Culture no. and treatment	F_1 soluble in anhydrous ether			F_2 soluble in hot alcohol and water			F_3 insoluble in alcohol, ether, and water		
	Percentage fraction	Percentage total leaf	Percentage total P in leaf	Percentage fraction	Percentage total leaf	Percentage total P in leaf	Percentage fraction	Percentage total leaf	Percentage total P in leaf
44. High N warm......	0.7369	0.0641	9.23	0.8683	0.2866	41.28	0.5898	0.3437	49.49
24. High N warm......	0.6847	0.0539	7.93	0.8211	0.2770	40.80	0.5960	0.3478	51.27
41. High P and N warm	0.7857	0.0819	10.26	1.3394	0.4368	54.69	0.4918	0.2800	35.05
108. High N cool.......	0.7739	0.0638	11.65	0.5352	0.1622	29.62	0.5239	0.3218	58.73
87. High N cool.......	0.7247	0.0627	10.99	0.5096	0.1417	24.80	0.5614	0.3565	64.21
104. High P and N cool..	0.7975	0.0612	9.37	0.9602	0.2907	44.50	0.4856	0.3012	46.13

TABLE XI

EFFECT OF TEMPERATURE UPON SOLUBILITY OF F_3 NITROGEN OF BARLEY LEAVES IN 1 PER CENT NaOH (MATERIAL DIGESTED WITH 1 PER CENT NaOH FOR 48 HOURS AT 37–40° C.)

Culture no. and treatment	Soluble nitrogen			Insoluble nitrogen		
	Percentage fraction	Percentage total leaf	Percentage total N in leaf	Percentage fraction	Percentage total leaf	Percentage total N in leaf
44. High N warm...........	0.910	0.5303	17.81	2.649	1.5440	51.85
24. High N warm...........	0.8570	0.4902	18.17	2.380	1.3892	51.43
41. High P and N warm.....	1.126	0.6411	18.50	2.927	1.6670	48.10
108. High N cool............	0.873	0.5361	20.93	2.478	1.5221	59.43
87. High N cool............	1.052	0.6681	26.03	2.263	1.4373	54.75
104. High P and N cool......	0.923	0.5726	22.07	2.406	1.4926	57.53

TABLE XII

EFFECT OF TEMPERATURE UPON SOLUBILITY OF F_3 PHOSPHORUS OF BARLEY LEAVES IN 1 PER CENT NaOH (MATERIAL DIGESTED WITH 1 PER CENT NaOH FOR 48 HOURS AT 37–40° C.)

Culture no. and treatment	Soluble phosphorus (by difference)			Insoluble phosphorus		
	Percentage fraction	Percentage total leaf	Percentage total P in leaf	Percentage fraction	Percentage total leaf	Percentage total P in leaf
44. High N warm...........	0.2292	0.1335	19.22	0.3606	0.2102	30.27
24. High N warm...........	0.1994	0.1163	17.13	0.3966	0.2315	34.10
41. High N and P warm.....	0.1959	0.1115	13.96	0.2959*	0.1685*	21.09
108. High N cool............	0.1132	0.0696	12.70	0.4107	0.2522	46.03
87. High N cool............	0.1807	0.1148	20.10	0.3807	0.2417	42.32
104. High N and P cool......	0.1666	0.1039	15.91	0.3190†	0.1979†	30.30

* Poor duplicates. † One analysis only, duplicate lost.

TABLE XIII

EFFECT OF TEMPERATURE UPON AMOUNT OF CELL WALL MATERIAL, ETC. $F_3-[(N \text{ in } F_3 \times 6.25)+(\text{starch in } F_3)]$; EXPRESSED AS PERCENTAGE OF TOTAL DRY WEIGHT OF LEAF

Culture no. and treatment	Cell wall material, etc.	Ratio of supporting tissue (cell walls, etc.) to all other plant substances, including water
44. High N warm	32.99	0.0470
24. High N warm	34.47	0.0525
41. High P and N warm	30.90	0.0367
Average warm	32.78	0.0454
108. High N cool	32.89	0.0539
87. High N cool	35.36	0.0581
104. High P and N cool	34.16	0.0558
Average cool	34.13	0.0559

TABLE XIV

EFFECT OF TEMPERATURE ON DISTRIBUTION OF PHOSPHORUS; SUMMARY TABLE

MATERIAL	No. 24, HIGH N, WARM		No. 87, HIGH N, COOL	
	Percentage total leaf	Percentage total P	Percentage total leaf	Percentage total P
Lipoid P, F_1	0.0539	7.94	0.0627	10.99
Phosphate P, F_2	0.2105	31.01	0.0714	12.80
Organic P, F_2	0.0665	9.80	0.0703	12.31
Phosphoprotein P, F_3	0.1411	20.80	0.0832	18.38
Nucleoprotein P, F_3	0.2067	30.45	0.2833	49.62
Total P	0.6787	....	0.5709	....

Results of chemical analysis

LIPIN FRACTION (F_1).—The results given in table V indicate that the temperature has very little effect upon the amount of lipins, except in the case of a high phosphorus supply, where the percentage of lipins is decidedly higher. This fact is possibly correlated with the higher percentage of phospho-lipin phosphorus in the entire leaf, as shown in the third column of table X, and the higher percentage of lipin N as shown in the third column of table IX. Since the proportion of lipin P is practically the same for both temperatures in the case of the high nitrogen series, these data lead to the conclusion that the lipin fraction is not an important growth determinant. The writer recognizes the desirability of more data.

ALCOHOL-WATER SOLUBLE FRACTION (F_2).—Table V shows a distinctly higher average percentage of these extractives at the higher temperature, although the order of difference is not large. When, however, the composition of this fraction is examined certain striking differences are noted. The high temperature leaves contain a much lower percentage of both total and reducing sugars (table VI) and a lower percentage of polysaccharides (table VII). The high temperature leaves contain about twice as much nitrogen (as determined by the unmodified Arnold-Gunning process) as do the low temperature leaves (table IX). In other words, the amount of active metabolic nitrogen, such as amino acids, polypeptides, and simpler water soluble proteins, is much higher at the higher temperature. The amount of nitric N is also higher at the higher temperature, as was indicated when the modified Arnold-Gunning process was used. The results of the nitric N determinations are not reported in this paper. The high temperature leaves also contain nearly twice the percentage of alcohol-water soluble phosphorus. Duplicate determinations on one set of samples (nos. 24 and 87) indicated that this difference was very largely due to the much higher percentage of inorganic phosphorus at the higher temperature. These results are appended, although it is recognized that more data are needed before any sweeping generalizations can be made. The Powick-Chapin method was used in this determination.

FIG. 16—Influence of temperature on maturation (photographed May 16): no. 12, "normal" fertilization (warm house); no. 74, "normal" fertilization (cool house).

	TOTAL P		INORGANIC P	
	Percentage of fraction	Percentage of entire leaf	Percentage of fraction	Percentage of entire leaf
No. 24	0.8211	0.2770	0.6240	0.2105
No. 87	0.5096	0.1417	0.2567	0.0714

FRACTION 3.—The higher amount of polysaccharides at the lower temperatures has been noted. Table V shows that the leaves grown at the lower temperature contain a distinctly higher average percentage of this fraction, although the order of difference is not large. Tables IX and X show that there is no important

FIG. 17

FIG. 18

FIGS. 17–18.—Fig. 17, influence of heavy N and heavy K on maturation (photographed May 16): no. 49, heavy N+heavy K (warm house); no. 112, heavy N+heavy K (cool house); comparison with other sets not shown indicate that K has no effect in causing difference; contrast heavy N cultures with normal N cultures of fig. 16; fig. 18, influence of heavy N and extra heavy P on maturation (photographed May 16): no. 63, heavy N+extra heavy P (warm house); no. 126, heavy N+extra heavy P (cool house); contrast with nos. 63 and 125 (same treatment) in fig. 15.

difference in the percentage of either N or P at the different temperatures. The amount of phosphoprotein phosphorus seems to run somewhat lower at the lower temperature (table VIII).

In five out of six cases (cf. column 3, table XII, with column 5, table VII) the amount of phosphorus in the NaOH extract exceeded the phosphorus precipitable from that extract by 1 per cent NaOH, indicating that either some organic phosphorus compounds had

been dissolved by the NaOH but had not been hydrolyzed, or that the magnesia mixture failed to give quantitative precipitations of the PO_4 ions under the conditions of the experiment.

Table IX reports a study of the solubility of the F_3 nitrogen in 1 percentage NaOH. The results are inconclusive, but are reported for the sake of completeness.

The calculations reported in table XIII are self-explanatory. It will be noted that the average proportions of framework material are considerably higher at the lower temperature. Microchemical examination of median cross-sections of the leaves and of the culms showed a greater degree of lignification of the xylem bundles at the lower temperature, a fact of added significance. Lignification of the vessels in the culm adds greatly to the strength of the stem. Referring to the enormous differences in growth habit as shown in the figures, we may conclude that the upright habit at the lower temperature is due to: (1) a greater proportion of culm to leaf; (2) a greater proportion of skeletal material in the leaf; (3) a greater degree of lignification of conductive tissues in both leaf and culm. These obvious anatomical facts, however, are but the expression of a difference in metabolic equilibria, especially the nitrogen-carbohydrate ratio.

Discussion

The experiments reported in this paper, as well as the results of earlier investigators, reopen the question as to just what is meant by an optimum germination temperature. The classical investigations of HABERLANDT on germination temperature place the optimum at the temperature which most quickly permits the emergence of the radicle and plumule; in fact, practically all germination studies have been based upon this as the optimum. These optimum temperatures, at least for the cereals, are evidently too high to insure a future normal development. The writer believes that the course of development is to a large extent predetermined at a very early stage in the development of the plant by the chemical equilibria within the seedling, especially the nitrogen-carbohydrate ratio. These equilibria within the plant, like chemical reactions *in vitro*, are conditioned by the temperature and concentrations of the reacting substances. It seems likely that a high temperature

and a high nitrogen supply at an early stage in the development of the barley plant so shifts the equilibrium toward excessive vegetation as to prevent the normal tendency toward reproduction. Some other factor must be altered, therefore, as, for example, the water supply, if such plants are to be thrown into reproduction.

An investigation of the nitrogen-carbohydrate ratio at a different stage in the development of seeds and seedlings furnished with varying concentrations of nitrogenous compounds will probably throw considerable light upon these questions.

Conclusions

1. The excessive leaf production in the high temperature barley is caused by the high concentration of nitrates in the nutrient supplied.

2. Nitrate nitrogen in the nutrient begins to affect the subsequent course of development at high temperatures at the time of germination, or at least at a very early stage in the development of the plant. The tendency to excessive vegetation thus inaugurated cannot be counteracted by the addition of phosphorus or potassium salts.

3. The effect of the nutrient supply is reflected in the composition of the active organ, the leaf. The following equations represent the main facts revealed by chemical analysis of the leaf:

High heat supply + high nitrogen supply in nutrient solution = high soluble nitrogen in leaf + low soluble carbohydrate = excessive vegetation and little culm formation.

Low heat supply + high nitrogen supply in nutrient solution = low soluble nitrogen in leaf + high soluble carbohydrate = normal vegetation and normal culm formation.

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